

-Supplementary Information-

**End-Functionalized Glycopolymers as Mimetics of Chondroitin Sulfate
Proteoglycans**

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General Methods

Unless otherwise stated, reactions were performed in flame-dried glassware under an argon atmosphere and using dry solvents. Solvents were dried by passage through an activated alumina column under argon. All other commercially obtained reagents were used as received unless otherwise noted. Thin layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm). Visualization of the developed chromatogram was performed by UV, cerium ammonium molybdate or ninhydrin stain as necessary. ICN silica gel (particle size 0.032 - 0.063 mm) was used for flash chromatography. Gel filtration chromatography (Sephadex G-15 and G-25 ultrafine) was used in order to achieve purification of the glycopolymers.

¹H NMR and proton decoupling experiments were recorded on a Varian Mercury 300 (300 MHz), Varian Inova 500 (500 MHz), or Varian Inova 600 (600 MHz) spectrometer and are reported in parts per million (δ) relative to CDCl₃ (7.26 ppm), CD₃OD (4.87 ppm), DMSO-*d*₆ (2.50 ppm), and D₂O (4.80 ppm). Data for the ¹H NMR spectra are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant in Hz, and integration. ¹³C NMR spectra were obtained on a Varian Mercury 300 (75 MHz) or Varian Inova 500

(125 MHz) spectrometer and are reported in terms of chemical shift. Mass spectra were obtained from the Protein/Peptide MicroAnalytical Laboratory and the Mass Spectrometry Facility at the California Institute of Technology.

2-(2-((2S)-Bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethanol

Exo-norbornene ethanol (90 mg, 0.72 mmol) and 2-[2-[(1,1-dimethylethyl)dimethylsilyl]oxy]ethoxy]ethyl-1-methanesulfonate (240 mg, 0.80 mmol)¹ were dissolved in DMF (2.5 ml) and stirred for 15 min. The temperature was lowered to -10 °C, and NaH (35 mg, 0.87 mmol) was slowly added in three portions. The reaction was allowed to warm up to room temperature (rt) and refluxed overnight at 60 °C. The reaction was lowered to 0 °C, quenched with a 1:1 mixture of ether/water (10 ml), extracted with EtOAc (3 x 25 ml), dried over MgSO₄, and concentrated under reduced pressure. Flash silica gel column chromatography (hexanes:EtOAc 20:1 → 5:1) afforded the desired silyl-protected product (90 mg) in 39% yield. ¹H NMR (500 MHz; CDCl₃): δ 6.00 (d, *J* = 25 Hz, 2H), 3.71 (t, *J* = 5.5 Hz, 2H), 3.60 – 3.28 (m, 8H), 2.72 (s, 1H), 2.69 (s, 1H), 1.65 – 1.62 (m, 1H), 1.26 – 1.21 (m, 2H), 1.19 – 1.14 (m, 1H), 1.05 – 1.01 (m, 1H), 0.83 (s, 9H), 0.00 (s, 6H); ¹³C NMR (125 MHz; CDCl₃): δ 147.1, 86.4, 83.0, 81.1, 80.7, 73.1, 55.3, 54.0, 51.9, 49.1, 40.0, 36.3, 28.7, 5.1. ESI MS: *m/z* calcd for [C₁₈H₃₄O₃Si + H]⁺: 327.2356, obsd 327.2356.

The product from previous step (4.9 g, 15 mmol) was dissolved in THF (50 mL) and the temperature was lowered to 0 °C. A solution of TBAF (1M in THF, 30 mL, 30 mmol) was added to the reaction mixture dropwise. The reaction was stirred for 2 h at 0 °C, diluted with EtOAc (15 mL), quenched with H₂O, and extracted with EtOAc (3 x 15 mL). The organic layers were combined, dried over MgSO₄, and concentrated under reduced pressure. Flash silica column chromatography (hexanes:EtOAc 5:1 → 1:1) afforded the desired product (2.5 g) in 79% yield. ¹H NMR (500 MHz, CDCl₃): δ 6.11 – 6.04 (m, 2H), 3.72 – 3.37 (m, 10H), 2.80 (s, 1H), 2.74 (s, 1H), 2.48 (m, 1H), 1.71 (m, 1H), 1.34 – 1.23 (m, 3H), 1.13 –

1.09 (m, 1H); ^{13}C NMR (125 MHz, CDCl_3): δ 136.9, 136.8, 76.4, 72.2, 70.7, 70.6, 62.1, 45.2, 43.9, 41.8, 39, 30. ESI MS: m/z calcd for $[\text{C}_{12}\text{H}_{20}\text{O}_3 + \text{H}]^+$: 213.1491, obsd 213.1483.

2-(2-(Bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-4,6-*O*-*p*-methoxybenzylidene-2-deoxy-2-trichloroacetamido-D-galactopyranoside 6

A mixture of donor **4**² (0.180 g, 0.164 mmol) and acceptor **5** (0.104 g, 0.492 mmol) was co-evaporated with toluene (3 x 3 mL) and dried under vacuum overnight. The mixture was dissolved in CH_2Cl_2 (2.1 mL), and activated 4Å powdered molecular sieves were added. The reaction was stirred at rt for 30 min, cooled to -75 °C, and then stirred for an additional 30 min. Trimethylsilyl trifluoromethanesulfonate (0.5 M in CH_2Cl_2 , 66 μL , 0.033 mmol) at -75 °C was added to the reaction dropwise. The reaction was warmed to -15 °C, stirred for 40 min, and quenched with triethylamine. The reaction mixture was filtered through Celite and concentrated to afford a yellow syrup. The product was purified by flash chromatography (25 % EtOAc:toluene) to afford **6** (0.130 g, 69.2 %) as a white solid. R_f = 0.40 (30 % EtOAc:toluene). ^1H NMR (500 MHz, CDCl_3): δ 7.88 – 7.80 (m, 4 H, ArH), 7.48 – 7.40 (m, 2 H, ArH), 7.37 (d, J = 8.5 Hz, 2 H, $\text{C}_6\text{H}_4\text{OMe}$), 7.30 (dd, J = 7.5, 7.5 Hz, 2 H, ArH), 7.28 (dd, J = 7.5, 7.5 Hz, 2 H, ArH), 6.84 (d, J = 9.0 Hz, 2 H, $\text{C}_6\text{H}_4\text{OMe}$), 6.84 (d, J = 7.0 Hz, 1 H, NH), 6.08 – 6.00 (m, 2 H, CH=CH), 5.46 (dd, J = 8.5, 8.5 Hz, 1 H, H-3'), 5.43 (s, 1 H, MeOPhCH), 5.39 (dd, J = 7.0, 7.0 Hz, 1 H, H-2'), 5.06 (d, J = 7.5 Hz, 1 H, H-1'), 5.04 (d, J = 8.0 Hz, 1 H, H-1), 4.63 (dd, J = 3.5, 11.0 Hz, 1 H, H-3), 4.33 (d, J = 4.5 Hz, 1 H, H-4'), 4.31 (dd, J = 8.5, 8.5 Hz, 1 H, H-4), 4.25 (d, J = 12.0 Hz, 1 H, H-6), 4.09 (d, J = 9.5 Hz, 1 H, H-5'), 4.02 (d, J = 11.5 Hz, 1 H, H-6), 3.97 – 3.90 (m, 1 H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.78 (s, 3 H, OCH_3), 3.77 (s, 3 H, OCH_3), 3.76 – 3.62 (m, 2 H, $\text{OCH}_2\text{CH}_2\text{O}$), 3.61 – 3.43 (m, 8 H, H-5, $\text{OCH}_2\text{CH}_2\text{O}$), 3.29 (td, J = 7.0, 2.0 Hz, 1 H, H-2), 2.75 (s, 1 H, CH of norbornene), 2.69 (s, 1 H, CH of norbornene), 1.68 – 1.58 (m, 1 H, CH of norbornene), 1.30 – 1.14 (m, 3 H, CH of norbornene), 1.00 – 1.18 (m, 1 H, CH of norbornene), 0.70 (s, 9 H, $\text{SiC}(\text{CH}_3)_3$), -0.09 (s, 3 H, SiCH_3), -0.25 (s, 3 H, SiCH_3); ^{13}C NMR (75 MHz,

CDCl₃): δ 168.84, 165.76, 165.27, 162.32, 160.07, 136.83, 136.78, 133.41, 133.36, 130.55, 130.06, 129.90, 129.61, 129.25, 128.53, 128.52, 127.69, 76.36, 76.22, 75.63, 75.59, 73.59, 73.58, 71.96, 70.84, 70.73, 70.59, 70.58, 70.43, 69.13, 68.65, 66.75, 55.48, 55.12, 52.76, 45.17, 43.80, 41.71, 38.90, 29.89, 25.58, 17.91, -4.24, -4.98. ESI MS: m/z calcd for [C₅₅H₆₈Cl₃NO₁₇Si + H]⁺: 1148.3, obsd 1148.7.

2-(2-(Bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl (methyl 2,3-di-O-benzoyl-4-O-tert-butyltrimethylsilyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-2-deoxy-2-acetamido- β -D-galactopyranoside

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Reduction of the trichloroacetamide group was performed using a procedure modified from Bélot *et. al.*³ Disaccharide **6** (0.058 g, 0.050 mmol) was dissolved in benzene (1.5 mL), and tributylstannane (80 μ L, 0.30 mmol) and 2,2'-azobisisobutyronitrile (1.6 mg) were added. After stirring at rt for 45 min, the reaction mixture was heated to 80 °C and stirred for an additional 3 h. The reaction was then cooled to rt and concentrated to afford a white solid. The product was purified by flash chromatography (50 \rightarrow 90% EtOAc:hexanes) to afford the desired acetamide (0.040 g, 77%) as a white solid. R_f 0.40 (EtOAc). ¹H NMR (500 MHz, CDCl₃): δ 7.88 – 7.82 (m, 4 H, ArH), 7.46 (d, J = 8.5 Hz, 1 H, ArH), 7.44 (d, J = 8.5 Hz, 1 H, ArH), 7.41 (d, J = 9.0 Hz, 2 H, C₆H₄OMe), 7.34 – 7.28 (m, 4 H, ArH), 6.84 (d, J = 9.0 Hz, 2 H, C₆H₄OMe), 6.07 – 5.99 (m, 2 H, CH=CH of norbornene), 5.52 (t, J = 9.0 Hz, 1 H, H-3'), 5.48 (t, J = 7.0 Hz, 1 H, NH of amide), 5.42 (s, 1 H, MeOPhCH), 5.34 (dd, J = 6.0, 9.0 Hz, 1 H, H-2'), 5.06 (d, J = 9.0 Hz, 1 H, H-1), 4.97 (d, J = 7.0 Hz, 1 H, H-1'), 4.71 (dd, J = 3.0, 11.0 Hz, 1 H, H-3), 4.37 (t, J = 9.0 Hz, 1 H, H-4'), 4.27 (d, J = 3.5 Hz, 1 H, H-4), 4.22 (dd, J = 1.0, 11.5 Hz, 1 H, H-6), 4.08 (d, J = 9.5 Hz, 1 H, H-5'), 4.00 (dd, J = 4.0, 12.0 Hz, 1 H, H-6), 3.95 – 3.88 (m, 1 H, OCH₂CH₂O), 3.77 (s, 3 H, CO₂CH₃), 3.75 (s, 3 H, PhOCH₃), 3.67 – 3.60 (m, 1 H, OCH₂CH₂O), 3.58 – 3.40 (m, 8 H, OCH₂CH₂O), 3.37 – 3.30 (m, 1 H, H-5), 3.28 (td, 1 H, J = 1.0, 9.0 Hz, H-2), 2.75 (s, 1 H, CH of norbornene), 2.67 (s, 1 H, CH of norbornene), 1.67 – 1.57 (m, 1 H, CH of norbornene), 1.51 (s, 3 H, NCOCH₃), 1.28 – 1.12 (m, 3 H, CH of norbornene), 1.00 – 1.06 (m, 1 H, CH of norbornene), 0.69 (s, 9 H, SiC(CH₃)₃), -0.09 (s, 3 H, SiCH₃), -

0.25 (s, 3 H, SiCH₃); ¹³C NMR (75 MHz, CDCl₃): δ 171.47, 168.84, 165.84, 165.01, 160.01, 136.83, 136.81, 136.73, 136.71, 133.40, 133.32, 130.76, 129.89, 129.85, 129.68, 129.53, 128.59, 128.52, 127.78, 113.54, 101.46, 100.73, 99.09, 99.07, 76.20, 75.93, 75.66, 75.60, 72.37, 70.74, 70.59, 70.40, 70.37, 70.74, 70.59, 70.40, 70.37, 69.29, 68.49, 66.61, 55.45, 54.51, 52.66, 45.15, 45.14, 43.77, 43.76, 41.68, 38.86, 38.85, 29.87, 29.87, 25.57, 23.39, 17.90, -4.27, -4.98. ESI MS: *m/z* calcd for [C₅₅H₇₁NO₁₇Si - H]⁺: 1044.4, obsd 1044.7.

The acetamide (0.050 g, 0.048 mmol) was dissolved in CH₃CN (600 μL) and H₂O (200 μL). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.032 g, 0.143 mmol) was added. The reaction was stirred at rt for 2 h, quenched with MeOH, and concentrated to yield a red solid. The product was purified on Sephadex LH-20 (50% CH₂Cl₂:MeOH), followed by silica gel chromatography (CH₂Cl₂/MeOH (100:1 → 100:3 *v/v*)), to afford the desired diol **7** as a white solid (0.038 g, 86%). ¹H NMR (500 MHz, CDCl₃): δ 7.88 – 7.86 (m, 4 H, ArH), 7.49 – 7.44 (m, 2 H, ArH), 7.35 – 7.31 (m, 4 H, ArH), 6.07 – 6.04 (m, 2 H, CH=CH of norbornene), 5.56 (t, *J* = 9.0 Hz, 1 H, H-3), 5.50 (m, 1 H, NH of amide), 5.31 (dd, *J* = 9.0, 7.5 Hz, 1 H, H-2), 4.96 (d, *J* = 8.5 Hz, 1 H, H-1'), 4.88 (dd, *J* = 7.5, 2 Hz, 1 H, H-1), 4.56 (dd, *J* = 10.5, 2 Hz, 1 H, H-3'), 4.29 (t, *J* = 9.0 Hz, 1 H, H-4), 4.08 (dd, *J* = 9.0, 1.0 Hz, 1 H, H-5), 4.05 (bs, 1 H, H-4'), 3.93 (m, 1 H, OCH₂CH₂O), 3.88 (m, 1 H, OCH₂CH₂O), 3.79 (s, 3 H, CO₂CH₃), 3.76 (m, 1 H, OCH₂CH₂O), 3.66 (m, 1 H, OCH₂CH₂O), 3.59 – 3.47 (m, 6 H, OCH₂CH₂O), 3.44 (m, 1 H, H-6), 3.30 (m, 1 H, H-6), 3.21 (m, 1 H, H-2'), 2.78 (s, 1 H, CH of norbornene),), 2.73 (s, 1 H, H-5'), 2.68 (s, 1 H, CH of norbornene), 1.66 – 1.60 (m, 1 H, CH of norbornene), 1.31 (d, *J* = 4.0 Hz, 3 H, NCOCH₃), 1.30 – 1.23 (m, 2 H, CH of norbornene), 1.22 – 1.17 (m, 1 H, CH of norbornene), 1.07 – 1.03 (m, 1 H, CH of norbornene), 0.71 (s, 9 H, SiC(CH₃)₃), -0.06 (s, 3 H, SiCH₃), -0.21 (s, 3 H, SiCH₃); ¹³C NMR (125 MHz, CDCl₃): δ 171.30, 168.54, 165.82, 165.13, 136.98, 136.93, 136.75, 136.72, 133.61, 133.46, 129.96, 129.91, 129.57, 129.30, 128.73, 128.60, 102.11, 99.36, 99.36, 78.82, 77.44, 76.49, 76.31, 76.28, 75.02, 73.95, 72.15, 70.86, 70.59, 70.45, 69.21, 68.75, 68.76, 62.96, 54.57, 52.94, 45.24, 45.20, 43.85, 43.83, 41.76, 41.74, 38.93, 38.91,

29.97, 29.94, 25.61, 23.09, 17.96, -4.23, -4.91. ESI MS: m/z calcd for $[C_{47}H_{65}NO_{16}Si + H]^+$: 928.4, obsd 928.1.

2-(2-(Bicyclo[2.2.1]hept-5-en-2-ylmethoxy)ethoxy)ethyl (methyl 2,3-di-*O*-benzoyl-4-*O*-*tert*-butyldimethylsilyl- β -D-glucopyranosyluronate)-(1 \rightarrow 3)-4,6-di-*O*-sodium sulfonato-2-deoxy-2-acetamido- β -D-galactopyranoside 3

To a solution of diol **7** (0.145 g, 0.156 mmol) in DMF (7 mL) was added sulfur trioxide trimethylamine complex ($SO_3 \cdot TMA$) (0.550 g, 3.96 mmol). The reaction mixture was stirred at 50 °C overnight and then cooled to rt. The product was purified on Sephadex LH-20 (50% CH_2Cl_2 :MeOH), followed by silica gel chromatography (5:2:1 MeOH:EtOAc:H₂O), to afford **3** (0.144 g, 84.7%) as a white solid. R_f 0.35 (5:2:1 MeOH:EtOAc:H₂O). 1H NMR (500 MHz, CD_3OD): δ 7.87 (d, J = 7.0 Hz, 2 H, ArH), 7.84 (d, J = 7.0 Hz, 2 H, ArH), 7.53 (d, J = 7.0 Hz, 1 H, ArH), 7.50 (d, J = 7.0 Hz, 1 H, ArH), 7.36 (dd, J = 8.0, 8.0 Hz, 4 H, ArH), 6.12 – 6.04 (m, 2 H, $CH=CH$ of norbornene), 5.60 (t, J = 9.0 Hz, 1 H, H-3'), 5.39 (t, J = 9.0 Hz, 1 H, H-2'), 5.10 (d, J = 7.5 Hz, 1 H, H-1'), 4.94 (s, 1 H, H-4), 4.50 – 4.35 (m, 3 H, H-1, H-6, H-4'), 4.22 – 4.28 (dd, J = 7.5, 10.5 Hz, 1 H, H-6), 4.17 (dd, J = 2.0, 9.5 Hz, 1 H, H-5'), 4.04 – 3.85 (m, 4 H, H-2, H-5, OCH_2CH_2O), 3.83 (s, 3 H, OCH_3), 3.72 – 3.64 (m, 1 H, OCH_2CH_2O), 3.60 – 3.45 (m, 7 H, OCH_2CH_2O), 3.42 – 3.33 (m, 1 H, OCH_2CH_2O), 2.79 (s, 1 H, CH of norbornene), 2.69 (s, 1 H, CH of norbornene), 1.70 – 1.58 (m, 1 H, CH of norbornene), 1.48 (d, J = 3.5 Hz, 3 H, $NCOCH_3$), 1.36 – 1.26 (m, 2 H, CH of norbornene), 1.26 – 1.8 (m, 1 H, CH of norbornene), 1.16 – 1.10 (m, 1 H, CH of norbornene), 0.73 (s, 9 H, $SiC(CH_3)_3$), -0.02 (s, 3 H, $SiCH_3$), -0.17 (s, 3 H, $SiCH_3$); ^{13}C NMR (75 MHz, CD_3OD): δ 173.59, 170.08, 167.38, 166.87, 137.93, 137.90, 137.69, 134.66, 134.54, 131.09, 131.03, 130.91, 129.64, 129.59, 129.54, 103.37, 103.12, 79.12, 77.42, 77.22, 77.18, 77.16, 74.19, 74.08, 72.01, 71.63, 71.52, 70.18, 68.83, 53.41, 52.77, 46.03, 46.01, 45.04, 45.03, 42.91, 42.90, 40.18, 40.17, 30.82, 30.80, 26.20, 22.92, 18.81, -3.93, -4.53. ESI MS: m/z calcd for $[C_{47}H_{65}NO_{22}S_2Si-H]^-$: 1086.3, obsd 1086.3.

Polymerization procedure and direct end-capping of polymers with a biotin terminating agent

In a typical polymerization experiment, a small vial was charged with CS monomer (**3** or **7**; 0.015 mmol) and a small stir bar under the flow of argon. The monomer was dissolved in 600 μL of $(\text{CH}_2\text{Cl})_2/\text{MeOH}$ (5:1 v:v), and the desired amount of 0.013 M of bis-pyridine catalyst **8** stock solution in $(\text{CH}_2\text{Cl})_2$ was added via syringe at rt. The reaction mixture was heated to 55 $^\circ\text{C}$ and stirred at this temperature for 5 ~ 30 min. At this point, TLC of the crude reaction mixture showed complete consumption of starting material. The reaction was quenched with ethyl vinyl ether (300 μL) to obtain polymers **9** or **10** as white precipitates. The solvent was removed *in vacuo* to obtain a solid precipitate, which was dissolved in minimal amount of $\text{CH}_2\text{Cl}_2:\text{MeOH}$ (10:1). The polymer was then precipitated by slowly adding this solution to 25 mL of hexanes in a 50-mL beaker. The hexanes was decanted to obtain the CS polymer as white precipitate in 87 – 92 % yield. The ^1H NMR of the crude reaction mixture showed the disappearance of the norbornene olefinic protons around 6 ppm, indicating completion of the polymerization reaction. The glycopolymers were characterized by ^1H NMR spectroscopy and gel permeation chromatography (GPC). GPC for compounds **9** and **10** was carried out in 0.2 M LiBr in DMF on two I-series Mixed Bed Low MW ViscoGel columns (Viscotek), connected in series with a DAWN EOS multiangle laser light scattering (MALLS) detector and an Optilab DSP differential refractometer (both from Wyatt Technology). The polymerization yield and Mw, Mn and PDI values for different CS monomers and different catalyst loadings are shown in **Table 1**. To install biotin at the end of the CS glycopolymers (**13** and **14**), biotin terminating agent solution (5 mg of **12** in 0.25 mL of $\text{MeOH}/(\text{CH}_2\text{Cl})_2$ (1:1)) was added to the reaction mixture instead of quenching the reaction with ethyl vinyl ether. GPC analysis was used to confirm completion of the polymerization reaction before addition of the biotin terminating agent. The reaction was stirred under argon at 55 $^\circ\text{C}$ for another 6 h, at which point ethyl vinyl ether (300 μL) was added. The work-up procedure was performed as described above, and the mixture of polymer and excess biotin terminating agent was directly used in the next step.

Protected CS-E polymers **9** and **10**

The polymerization procedure above was used to synthesize glycopolymers **9** and **10** in 87% and 92% yield, respectively. ^1H NMR (500 MHz, DMSO-*d*6) δ 7.97 – 7.77 (m, 4 H, ArH), 7.58 – 7.43 (m, 2 H, ArH), 7.43 – 7.29 (m, 4 H, ArH), 5.58 (bs, 1 H, H-3'), 5.37 – 5.02 (m, 4 H, H-2', H-1', CH=CH of norbornene), 4.75 (s, 1 H, H-4), 4.56 – 4.40 (m, 1 H, H-1), 4.38 – 3.98 (m, 4 H, H-4', H-5', H-6), 3.98 – 3.28 (m, 13 H, H-2, H-3, H-5, OCH₂CH₂O), 3.77 (s, 3 H, COOCH₃), 2.87 – 2.40 (m, 2 H, CH of norbornene), 1.90 – 1.20 (m, 5 H, CH of norbornene), 1.43 (s, 3 H, COCH₃), 0.74 (s, 9 H, SiC(CH₃)₃), -0.01 (s, 3 H, SiCH₃), -0.18 (s, 3 H, SiCH₃).

Polymer deprotection procedure

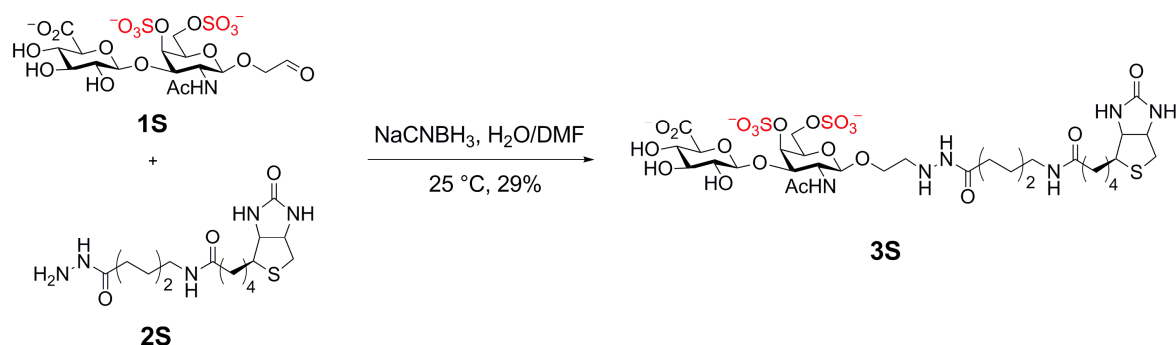
The protected CS polymers (0.015 mmol) were dissolved in pyridine (120 μL), THF (120 μL), and H₂O (30 μL). The reaction was cooled to 0 °C, and HF•pyridine (35 μL) was added. After stirring at 0 °C for 1 h and at rt overnight, the reaction was quenched with methoxy trimethylsilane to obtain a brown precipitate. The precipitate was filtered, washed with ether (4 times) and subjected to the saponification conditions. Saponification was performed in a manner similar to a procedure from Lucas *et. al.*⁴ To a solution of the alcohol in THF (400 μL) and H₂O (200 μL) at 0 °C were added 1 M aq. LiOH (100 μL) and 30% H₂O₂ (50 μL). The reaction was stirred at 0 °C for 1 h and at rt for 12 h. At this time, 4 M NaOH (160 μL) and MeOH (400 μL) were added. The reaction was stirred for another 24 h and then neutralized with Amberlyst IR-120 resin, filtered through 0.2 μm syringe filter (excess biotin terminating agent can be removed by filtration because only the polymers are soluble in water), and lyophilized to afford an orange solid. The product was purified by Sephadex G-25 or G-15 (0.9 % NaCl in H₂O) and desalted with Sephadex G-25 (100% H₂O) to afford **2**, **11** (71 – 80 % yield), **1** (74%), or **15** (55%) as white solids upon lyophilization. The yields of **2** and **11** are reported for 2 steps – desilylation and saponification; the yields

of **1** and **15** are reported for 3 steps – polymerization, desilylation, and saponification. For compounds **1** and **15**, GPC was carried out in 100 mM NaNO₃ and 200 ppm NaN₃ in water on an OHpak SB – 804 HQ column (Shodex), which was connected in series with a miniDAWN TREOS MALLS detector and Optilab rEX differential refractometer (both from Wyatt Technology). The dn/dc values were obtained for each injection assuming 100% mass elution from the column using dextran (10K and 40K) as a calibration standard to confirm complete mass recovery. The polymerization yield and Mw, Mn and PDI values for different CS monomers and different catalyst loadings are shown in **Table 1**.

CS-E polymers 2 and 11: ¹H NMR (500 MHz, D₂O): δ 5.60 – 5.10 (m, 2 H, CH=CH), 4.80 (s, 1 H, H-4), 4.55 (bs, 1 H, H-1), 4.43 (d, *J* = 8.0 Hz, 1 H, H-1'), 4.25 (d, *J* = 8.0 Hz, 1 H, H-6), 4.14 (dd, *J* = 9.0, 9.0 Hz, 1 H, H-6), 4.09 – 3.80 (m, 4 H, H-2, H-3, H-5, OCH₂CH₂O), 3.80 – 3.20 (m, 13 H, H-2', H-3', H-4', H-5', OCH₂CH₂O), 2.90 – 2.60 (bs, 1 H, CH of norbornene), 2.55 – 2.20 (bs, 1 H, CH of norbornene), 1.90 (s, 3 H, COCH₃ GalNAc), 2.20 – 1.60 (bs, 2 H, CH of norbornene), 1.70 – 1.40 (bs, 2 H, CH of norbornene), 1.30 – 0.90 (m, 1 H, CH of norbornene).

Biotinylated CS-E polymer 1: ¹H NMR (500 MHz, D₂O): δ 7.54 – 7.36 (m, 0.1 H, C₆H₅ of polymer chain terminal), 6.55 – 6.4 (bs, 0.017 H, NH of biotin), 5.55 – 5.35 (m, 2 H, CH=CH), 4.88 (s, 1 H, H-4), 4.63 (bs, 1 H, H-1), 4.53 (d, *J* = 7.5 Hz, 1 H, H-1'), 4.34 (d, *J* = 9.5 Hz, 1 H, H-6), 4.24 (m, 1 H, H-6), 4.15 – 4.05 (m, 4 H, H-2, H-3, H-5, OCH₂CH₂O), 3.85 – 3.20 (m, 13 H, H-2', H-3', H-4', H-5', OCH₂CH₂O), 3.05 – 2.85 (br s, 1 H, CH of norbornene), 2.65 – 2.40 (bs, 1 H, CH of norbornene), 2.07 (s, 3 H, COCH₃ GalNAc), 2.20 – 1.80 (bs, 2 H, CH of norbornene), 1.80 – 1.50 (bs, 2 H, CH of norbornene), 1.30 – 1.10 (m, 1 H, CH of norbornene).

Biotinylated unsulfated polymer 15: ¹H NMR (500 MHz, D₂O): δ 5.60 – 5.20 (m, 2 H, CH=CH), 4.60 – 4.50 (m, 2 H, H-1, H-1'), 4.21 (bs, 1 H, H-4), 4.10 – 4.00 (m, 2 H, H-2, H-6), 3.89 (m, 1 H, H-6), 3.85 – 3.48 (m, 14 H, H-3, H-5, H-4', H-5', OCH₂CH₂O, OCH₂), 3.42 – 3.34 (m, 2 H, H-2', H-3'), 3.10 – 2.70 (bs, 1 H, CH of norbornene), 2.70 – 2.40 (bs, 1 H, CH of norbornene), 2.05 (s, 3 H, COCH₃ GalNAc), 2.30 – 1.85 (bs, 2 H, CH of norbornene), 1.85 – 1.50 (bs, 2 H, CH of norbornene), 1.40 – 1.00 (m, 1 H, CH of norbornene).



***N*-(6-(2-(ethyl- β -D-glucopyranosyluronate-(1 \rightarrow 3)-4,6-di-*O*-sulfonato-2-deoxy-2-acetamido- β -D-galactopyranosyl)hydrazinyl)-6-oxohexyl)-5-(2-oxohexahydro-1H-thieno[3,4-*d*]imidazol-4-yl)pentanamide (3S)**

The biotin hydrazide compound **2S** (0.5 mg, 1.2 μmol ; Thermo Scientific) was dissolved in DMF (60 μL) and added to a solution of aldehyde **1S**⁵ (0.5 mg, 0.8 μmol) in H₂O (60 μL). The reaction was stirred at rt for 3 h. At this time, NaCNBH₃ (1 mg, 16 μmol) in H₂O was added. The reaction mixture was passed over an LH-20 column (3:1 MeOH:H₂O) and lyophilized. The resulting product was purified using a SepPak C18 column (500 mg resin, 100% H₂O to 1:9 CH₃CN:H₂O) to afford the desired product **3S** as a white solid (0.245 mg, 29.4%). ¹H NMR (600 MHz, D₂O): δ 4.85 (m, 1 H, H-4'), 4.63 (m, 1 H, CH of biotin), 4.58 (m, 1 H, H-1'), 4.51 (d, J = 7.8 Hz, 1 H, H-1), 4.45 (m, 1 H, CH of biotin), 4.33 (m, 1 H, OCH₂CH₂NH), 4.23 (m, 1 H, OCH₂CH₂NH), 4.11 (m, 2 H, OCH₂CH₂NH), 3.79 – 3.73 (m, 2 H, H-2', H-3'), 3.61 – 3.59 (m, 2 H, H-6', H-6'), 3.57 – 3.53 (m, 2 H, H-5', H-4), 3.49 (m, 1 H, H-5), 3.44 – 3.35 (m, 3 H, H-2, H-3, CH of biotin), 3.26 – 3.18 (m, 3 H, CH of biotin, CH₂NH), 3.04 – 3.01 (m, 1 H, CH of biotin), 2.31 – 2.22 (m, 4 H, C(O)CH₂), 1.93 (s, 3 H, NCOCH₃), 1.70 – 1.65 (m, 2 H, CH₂ of linker), 1.64 – 1.56 (m, 2 H, CH₂ of linker), 1.54 – 1.51 (m, 6 H, CH₂ of linker), 1.46 – 1.41 (m, 2 H, CH₂ of linker). ESI MS: m/z calcd for [C₃₂H₅₂N₆Na₂O₂₁S₃-H]⁻: 997.2, obsd 997.4.

Determination of glycopolymer concentrations

Concentrations of the glycopolymers were determined by measuring their relative uronic acid content using the carbazole reaction.⁶ Briefly, the acid borate reagent (1.5 mL of 0.80 g sodium tetraborate, 16.6

mL H₂O, and 83.3 mL H₂SO₄) was added to 15-mL glass test tubes. The glycopolymers (5 μ L of a 10 mg/mL stock in 3.5 M NaCl) were added and the solution placed in a boiling H₂O bath for 10 min. Following addition of the carbazole reagent (50 μ L of 0.1% w/v carbazole in 100% EtOH), the solution was boiled for 15 min. The absorbance was read at 530 nm and compared to a D-glucuronolactone standard in H₂O.

Biotin quantification

Incorporation of biotin into the polymers was quantified using the Fluorescence Biotin Quantitation Kit (Thermo Scientific). Briefly, the biotinylated polymers were dissolved in PBS, and DyLight Reporter (fluorescent avidin and HABA premix) was added to the biotinylated samples and a range of diluted biocytin standards. The avidin in this reporter fluoresces when the weakly interacting HABA (4'-hydroxyazobenzene-2-carboxylic acid) is displaced by the biotin. The amount of biotin in the polymer samples was then determined by comparing the sample's fluorescence to the biocytin standard curve. The amount of biotin in the sample was compared to the predetermined polymer concentration to determine the extent of biotinylation (20% for compound **1** and 8% for compound **15**). Alternatively, ¹H NMR spectroscopy was used to evaluate the percentage of biotinylation for **1** by comparing the integral values of the phenyl end groups ($\delta \sim 7.5$ ppm) derived from Grubbs' catalyst with the integral of NH proton of the biotin group ($\delta \sim 6.5$ ppm). A relaxation delay of 10 s was used to ensure precise integral values. NMR spectra were taken on a 500 MHz spectrometer for high resolution. According to NMR analysis, approximately 42% biotin incorporation was observed. However, the low S/N ratio and the high relative ratio of the monomer peaks relative to the polymer backbone and biotin peaks ($\sim 86:1$) reduced the accuracy of analysis by ¹H NMR.

Microarray assays

Carbohydrate microarrays were generated by spotting stock solutions of polymers **1** and **15** in PBS (1 nL) onto streptavidin coated slides (Xenopore) using a Microgrid II arrayer (Biorobotics; Cambridge, UK) at rt and 50% humidity. The concentrations of the stock solutions, which ranged from 500 nM to 50 μ M, were determined using the carbazole assay as described above and were corrected for the percentage of biotinylation. A given concentration of each polymer was spotted ten times at different positions on the array. A boundary was created around the polymer spots on the slides using a hydrophobic slide marker (Super Pap Pen, Research Products International), and the slides were blocked with 10% fetal bovine serum (FBS) in PBS (pH 7.4, unless otherwise indicated) with gentle rocking at 37 °C for 1 h, followed by a brief rinse with PBS. Human GDNF (Peprotech) was reconstituted in 1% Triton X-100 in PBS, added to the bound region on the slides at a concentration of 2 μ M (100 μ L), and incubated at rt for 3 h. The slides were briefly rinsed three times with PBS, and then incubated with a rabbit anti-human GDNF antibody (Peprotech; 1:1000 in 1% Triton X-100 in PBS) for 1 h at rt. The slides were again rinsed with PBS, and then incubated with an anti-rabbit antibody conjugated to Cy3 (Jackson ImmunoResearch; 1:5000 in PBS) for 1 h in the dark with gentle rocking. After rinsing two times with PBS and once with H₂O, the microarray was analyzed at 532 nm using a GenePix 5000a scanner, and fluorescence quantification was performed using GenePix 6.0 software. Binding of the anti-CS-C (2D5-1D2) and anti-CS-E (2D11-2A10) antibodies^{2, 5} was evaluated as described above using 100 μ L of a 10 μ g/mL (or ~70nM) solution of the antibody and a goat anti-mouse IgG secondary antibody conjugated to Cy3 (Jackson ImmunoResearch; 1:5000 in PBS). The experiments were performed in duplicate, and data representing the average of 20 spots per concentration were shown. Error bars represent the standard error of the mean.

Surface plasmon resonance

SPR experiments were performed on a Biacore T100 using CM5 sensors and HBS-EP+ buffer. Streptavidin-conjugated surfaces were prepared using the standard amine-coupling protocol with 1 μ M streptavidin in 10 mM sodium acetate buffer, pH 5.0 on all four flow cells. CS polymers **1** and **15** or the monovalent disaccharide **3S** (0.5 μ g/ml in HBS-EP+) were conjugated to flow cells 2 and 4, respectively, by a series of three 20–60s injections at 30 μ L/min, until the baseline response increased by ~25 RUs. Kinetic analyses were performed at 50 μ L/min with a 240 s association and 800 s dissociation, followed by a 30 s injection of 2.5 M MgCl_2 to regenerate the surface. Three startup injections of HBS-EP+ were performed before injecting GDNF (2, 1, 0.5, 0 nM) for both flow cells 2 (with 1 as reference), and 4 (with 3 as reference). To measure the K_D of the interaction with GDNF and glycopolymer **1**, GDNF (64, 32, 16, 8, 4, 2, 1, 0.5, 0.25, 0 nM) was injected as described above. The response at equilibrium was measured using Biacore T100 Evaluation software and plotted against the GDNF concentration. The K_D was determined by fitting the data to the Langmuir equation

$$R_{eq} = \frac{R_{max} \times [\text{GDNF}]}{K_D + [\text{GDNF}]}.$$

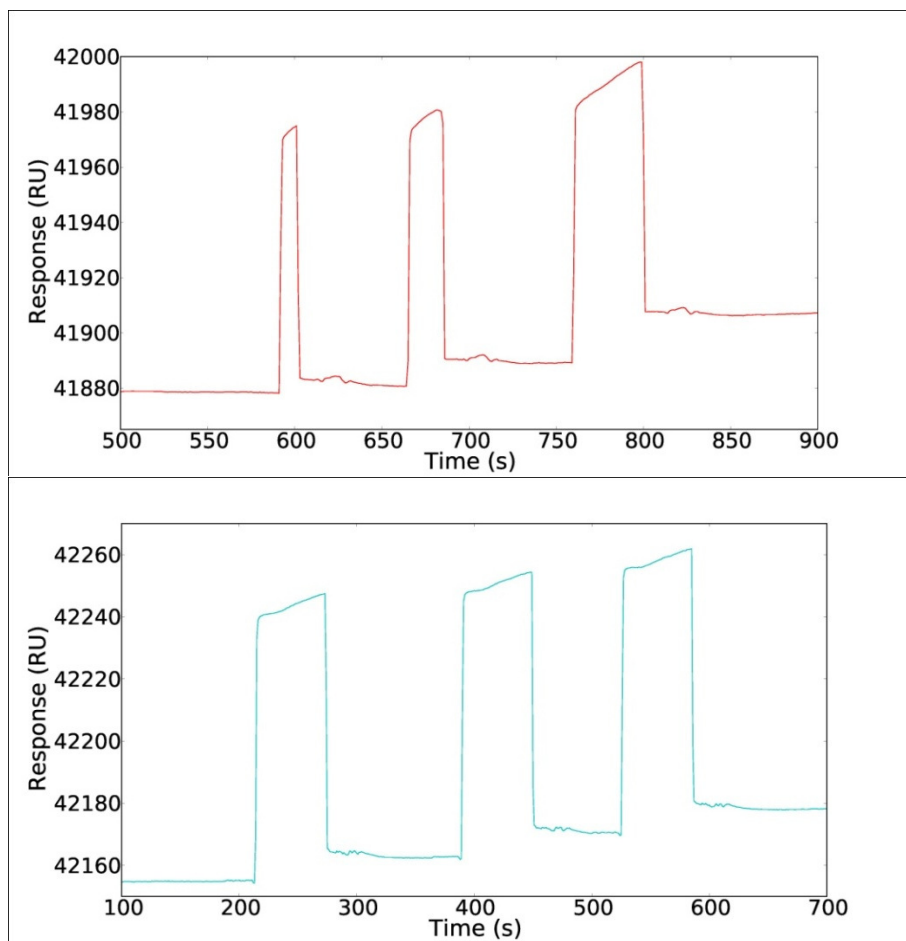


Figure S1. Sensorgram traces of the capture of polymers **1** (top) and **15** (bottom).

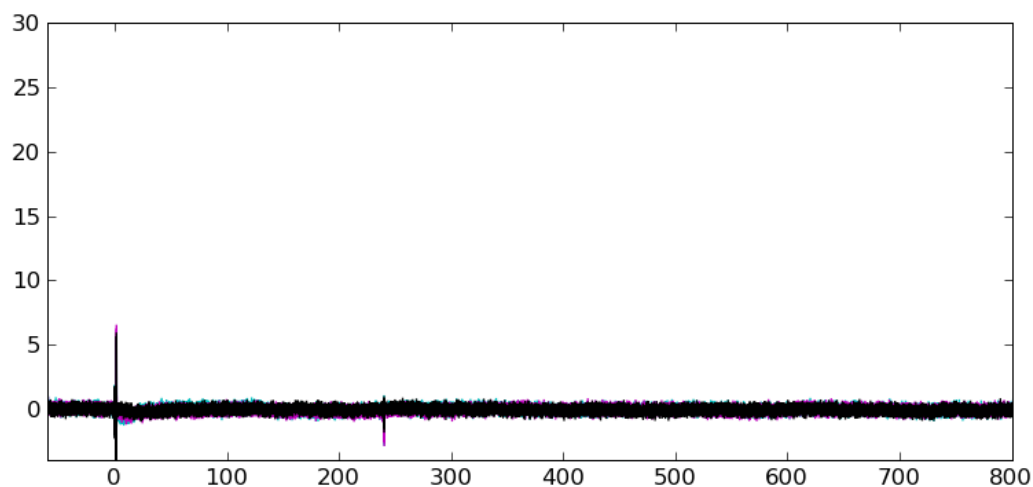


Figure S2. SPR sensorgram of the interaction between GDNF and the monovalent, biotinylated CS-E disaccharide **3S** at various GDNF concentrations (2 nM, cyan; 1 nM, magenta; 0.5 nM, black). No significant binding to the monovalent CS-E disaccharide was observed.

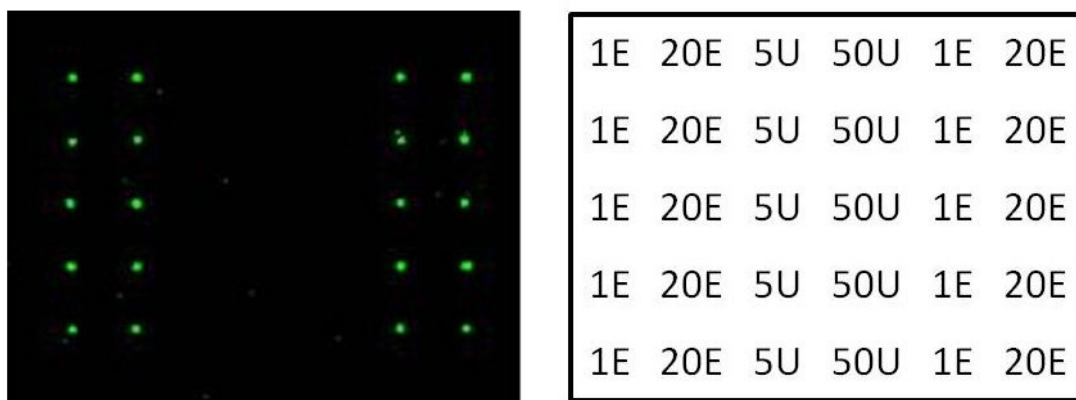
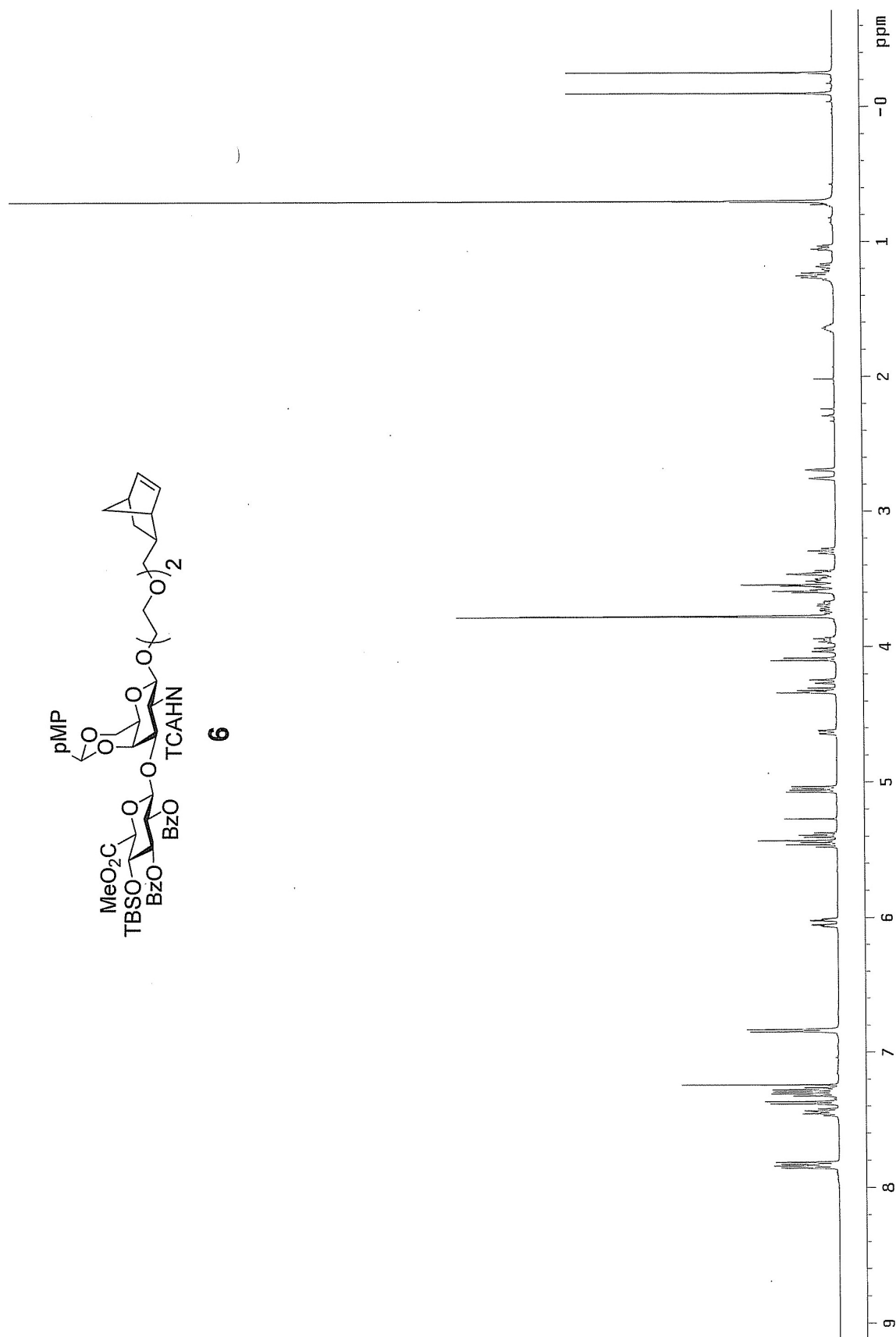
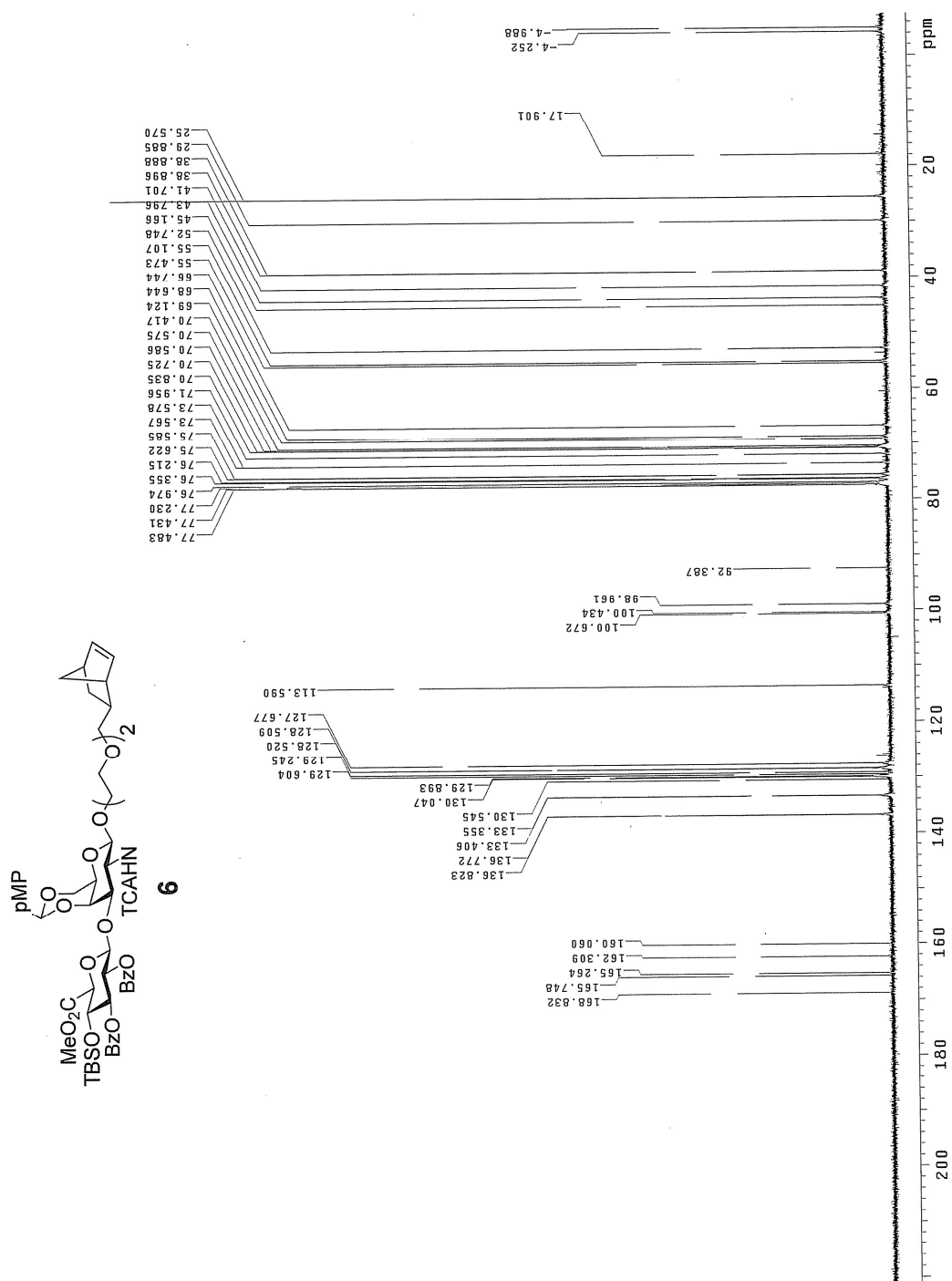
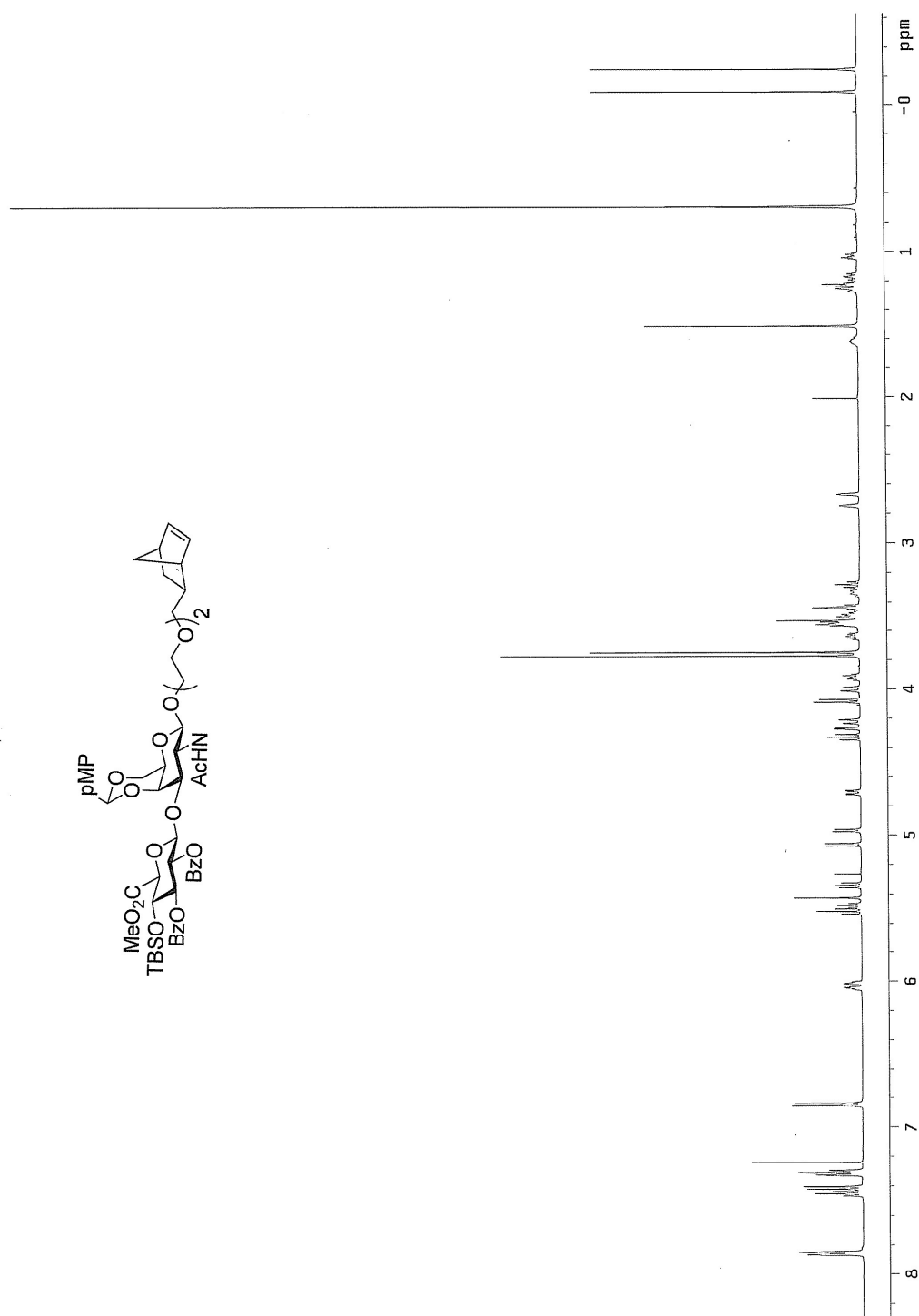
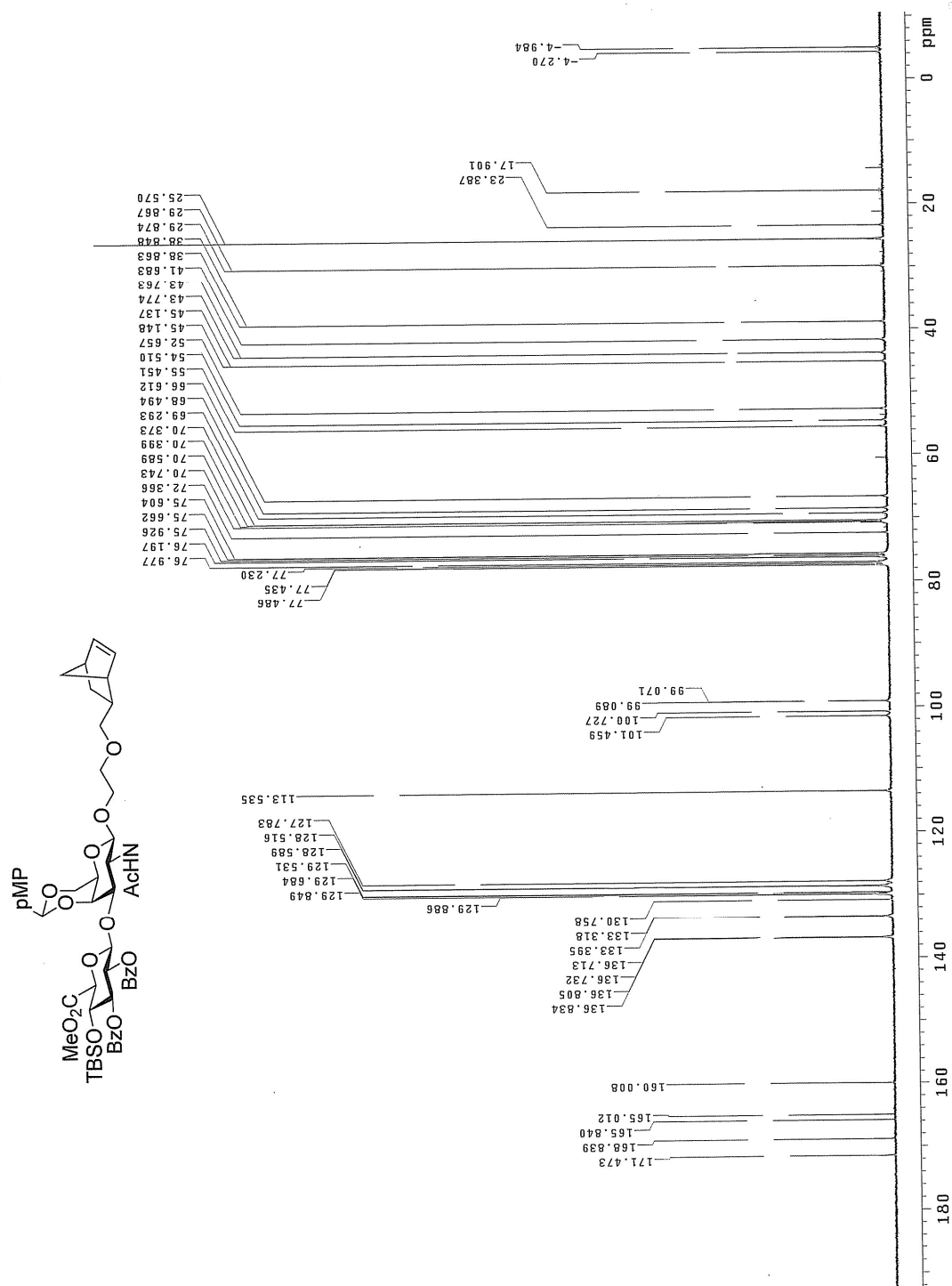


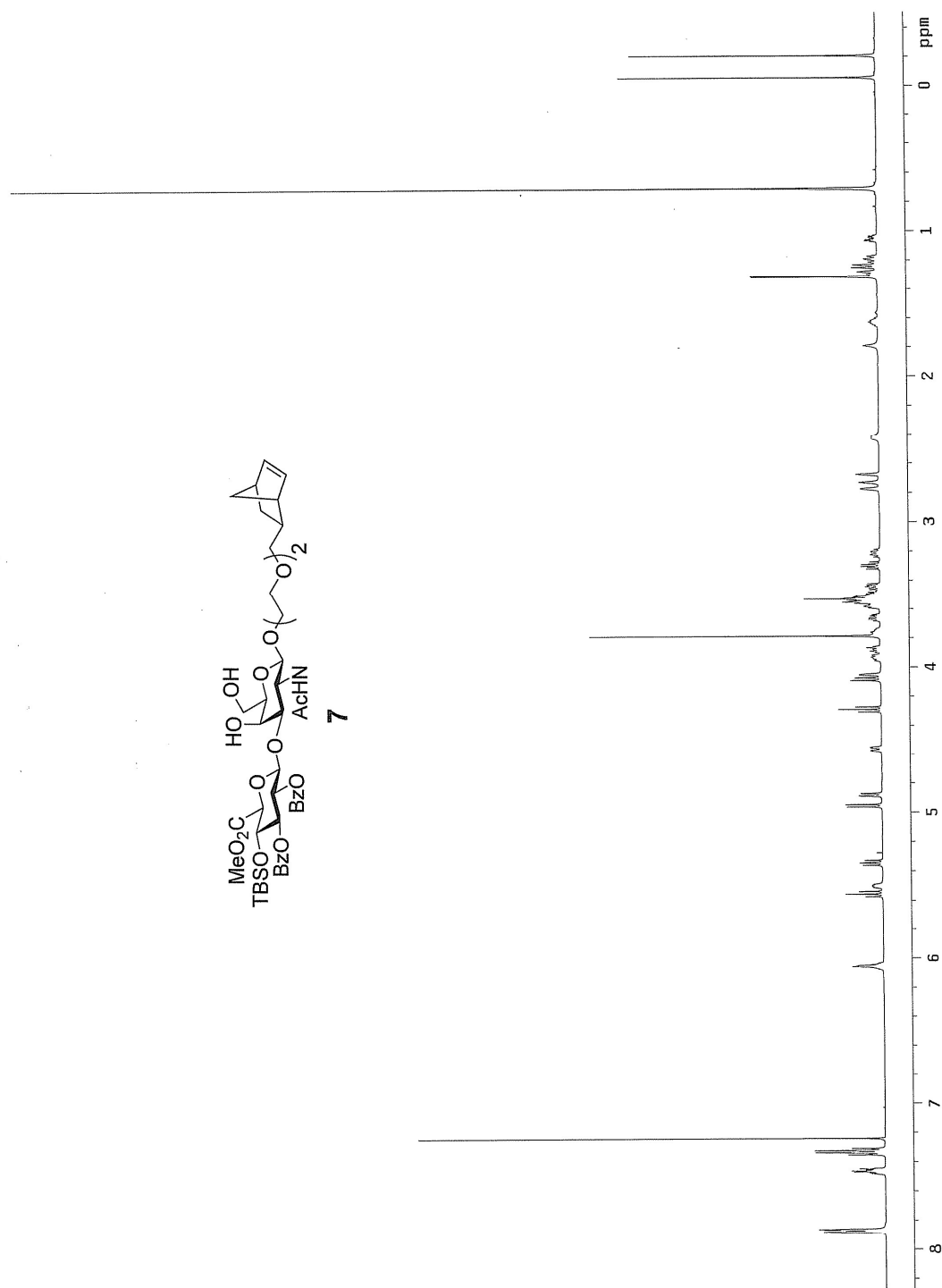
Figure S3. Representative portion of the microarray, illustrating spot morphology and fluorescence intensity after incubation with the CS-E antibody. Note that only a small portion of the microarray is shown. The panel on the right indicates the corresponding polymers and their concentrations for each spot shown. Values are in μM . E = CS-E polymer (polymer **1**), U = Unsulfated polymer (polymer **15**).

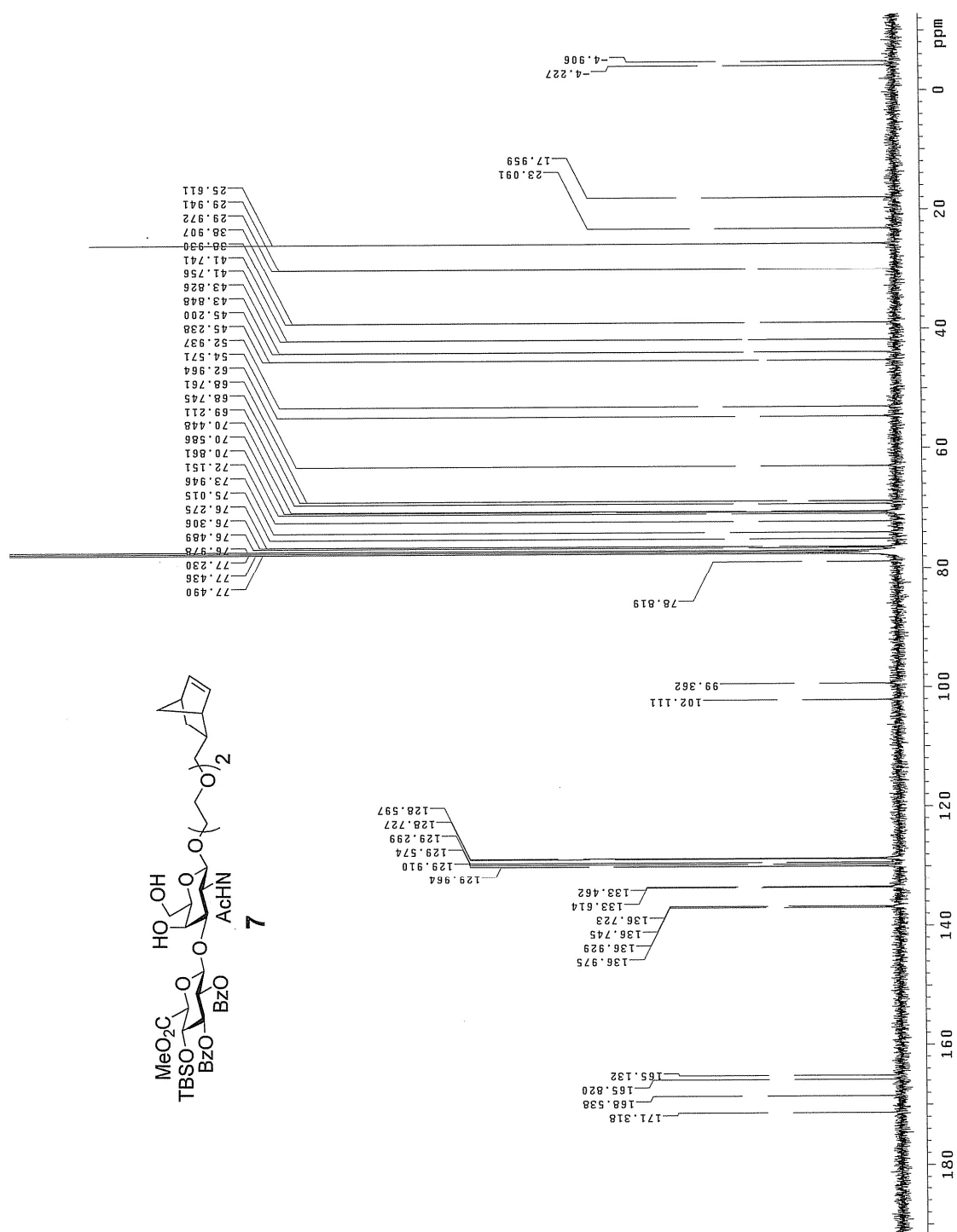


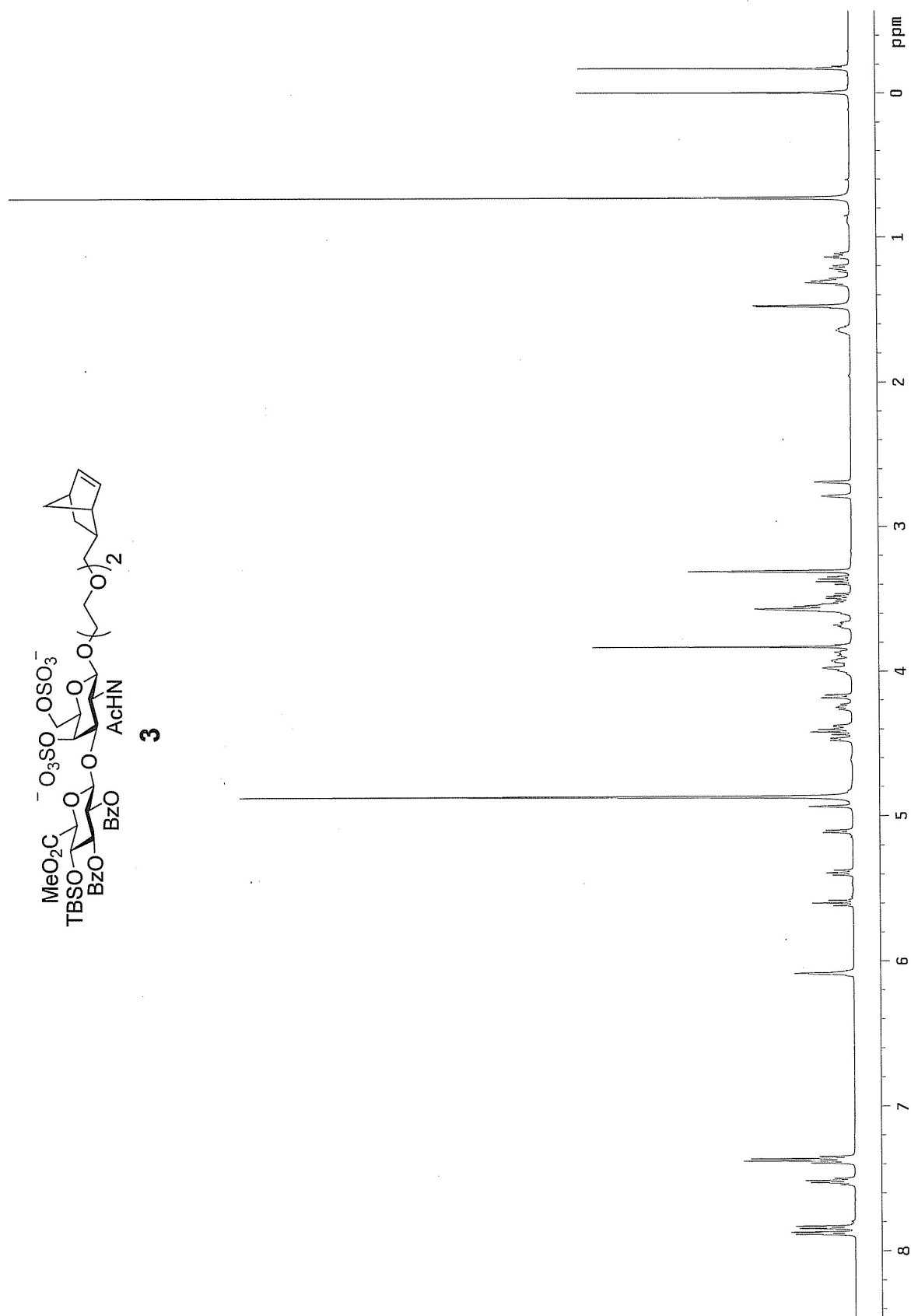




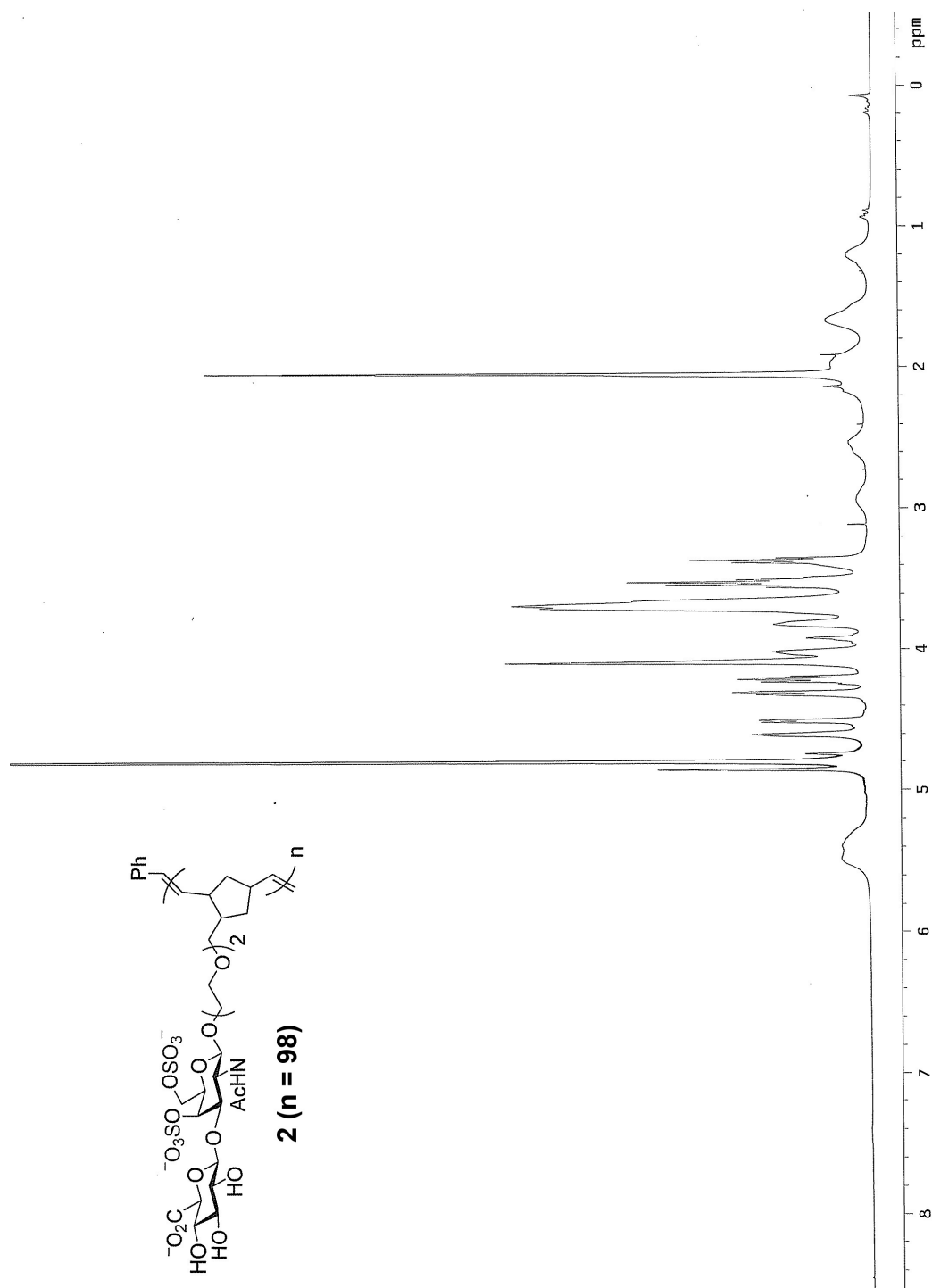


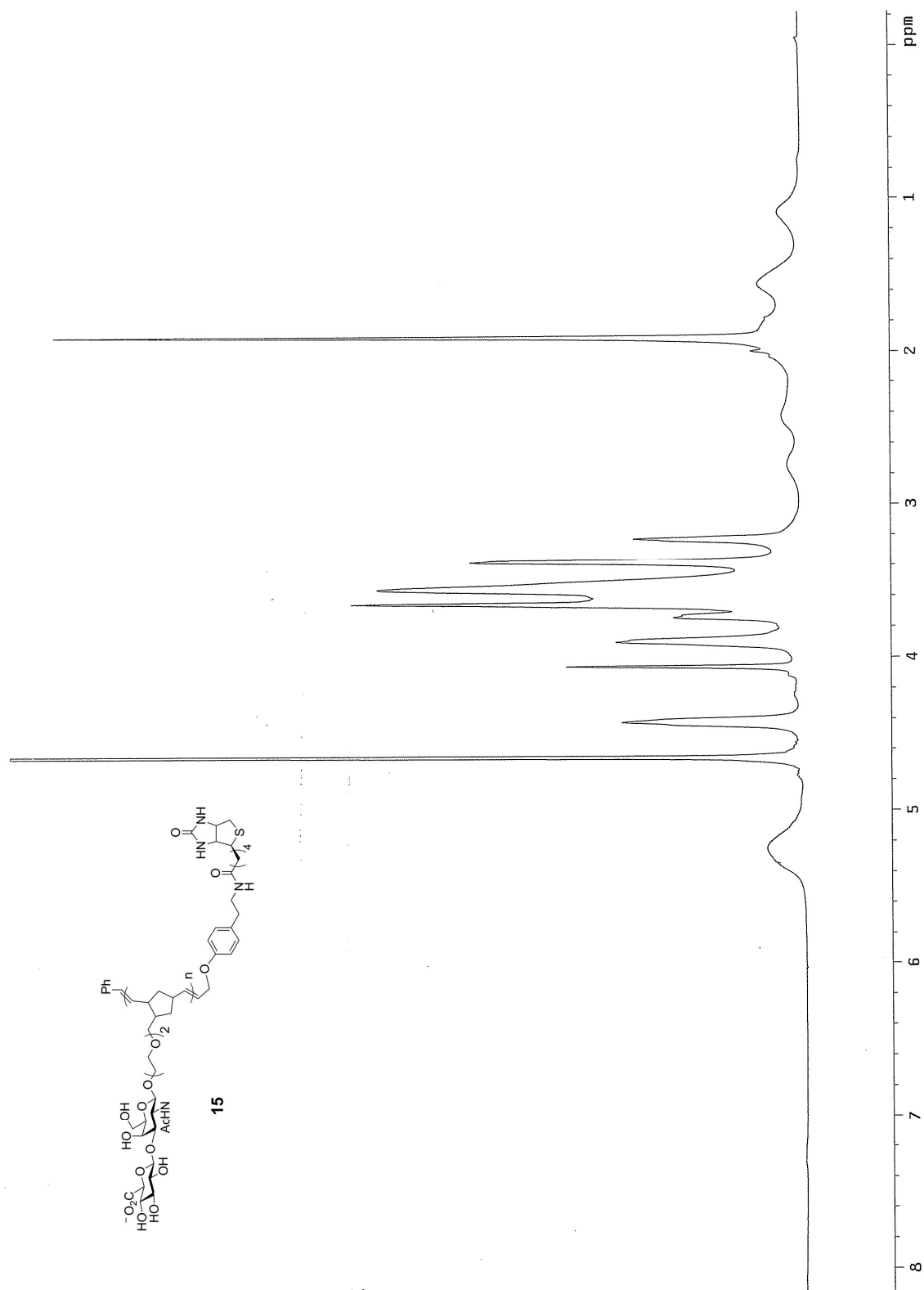


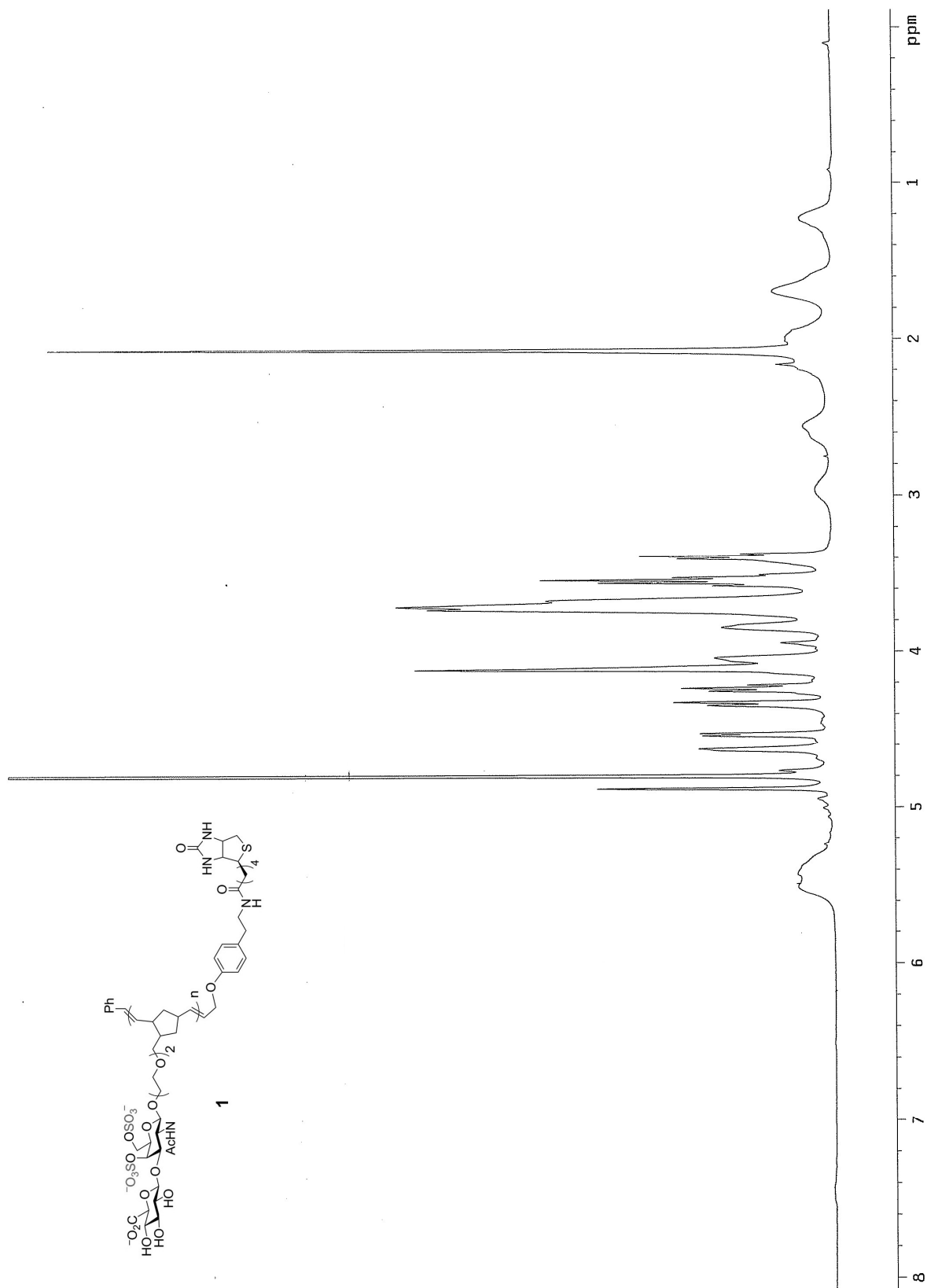












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